STABILITY CONSTANTS OF HBED WITH VARIOUS METAL IONS

A Thesis

by

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ABSTRACT

Stability Constants of HBED with Various Metal Ions. (December 1990)
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An investigation of the stability constants of the ligand N,N'-bis(2-hydroxylbenzyl)-ethylenediamine-N,N'-diacetic acid (HBED) with the metal ions Zn(II), Co(II), Ni(II), Cu(II), Gd(III), In(III), Fe(III), and Ga(III) is reported.

A combination of potentiometric titration and spectrophotometric titration at varying high -log[H]'s was used to determine the protonation constants for HBED. Potentiometric titrations of 1:1 metal:HBED solutions were used to obtain stability constants and metal chelate protonation constants for HBED with Zn(II), Co(II), Ni(II), Gd(III), and In(III), as well as the metal chelate protonation constants for Cu(II) and Fe(III). Spectrophotometric titrations at varying low -log[H]'s were used to obtain the stability constants for HBED with Cu(II) and Fe(III). The stability constant of HBED with Ga(III) was obtained by spectrophotometric titration of a 1:1 Fe(III):HBED solution with Ga(III).

The calculated values for the stability constants of HBED with Fe(III) and Ga(III) are two log units lower than stability constants reported earlier. The calculated stability constant for HBED with In(III) differs from an earlier reported value by twelve log units. Reasons for these differences are discussed.

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INTRODUCTION

Radiopharmaceuticals and NMR Contrast Agents

The importance for the determination of the stability constants for the ligand N,N'-bis(2-hydroxybenzyl)-ethylenediamine-N,N'-diacetic acid (HBED) with various metal ions is that it has the potential to form highly stable complexes with $^{67,68}Ga(III)$, $^{111}In(III)$, Fe(III), and Gd(III). These metal ions are potentially useful as radiopharmaceuticals and/or paramagnetic nuclear magnetic resonance (NMR) contrast agents.

One study demonstrated that $^{67,68}Ga(III)$ and $^{111}In(III)$ complexes of HBED and of N,N'-bis(pyridoxyl)ethylenediamine-N,N'-diacetic acid (PLED) are successful in imaging the kidneys of Sprague Dawley rats, while $^{67,68}Ga(III)$ and $^{111}In(III)$ complexes of N,N'-bis(2-hydroxy-3,5-dimethylbenzyl)-ethylenediamine-N,N'-diacetic acid (Me_4HBED) and of N,N'-bis(2-hydroxy-3-methyl-5-tertiary butyl)-ethylenediamine-N,N'-diacetic acid (t-BuHBED) were successful in radioimaging the liver of Sprague Dawley rats (Figure 1)[1]. Additionally, $^{111}In(III)$ labelled neutral phospholipids were found to image the liver and spleen.[2] Complexes of Fe(III) are effective NMR contrast agents because Fe(III) has five unpaired electrons.[3,4] Currently, Gd(III) complexes have been studied as NMR contrast agents to a greater extent than Fe(III), because of the greater

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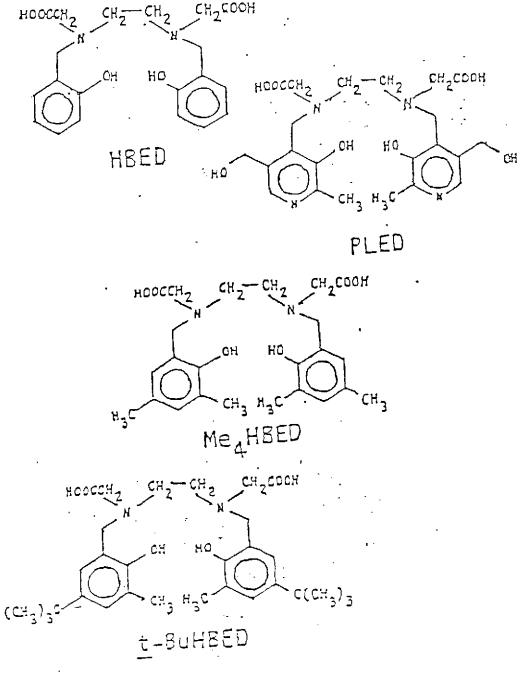


Figure 1. Structural formulas for HBED, PLED, Me_4HBED, and t-BuHBED.[1]

effectiveness of Gd(III), with seven unpaired electrons. Several studies have found the Gd(III) complex of diethylenetriaminepentaacatic acid (DTPA) to be a useful NMR contrast agent (Figure 2).[5,6,7] recently, a complex of Gd(III) with the macrocyclic ligand tetraazacyclodecane-tetraacetic acid (DOTA) was found to be an effective NMR contrast agent for kidney imaging.[8]

Complexes of $^{67,68}Ga(III)$, $^{111}In(III)$, Fe(III), and Gd(III), not simply the salts of these metals, are required for radioimaging and NMR imaging. If only the metal ions were used, they would be complexed by serum transferrin in vivo. Coordination of Ga(III) to transferrin is known to inhibit both the B-lymphocyte proliferation and the immunoglobulin production that are stimulated by the T-independent Epstein Barr virus (Figure 3).[10] If Ga(III) is allowed to complex with transferrin in vivo, then, there is evidence that at least one consequence wold be that immuno-globulin production would be reduced. Serum transferrin, the primary biological transport protein for iron, normally is only about 30% saturated with iron and thereby retains a high capacity to complex each other metal ions.[10] Because the conditional stability constant for transferrin for transferrin ($[GaL^*]/([Ga][L^*])$) at its two binding sites at physiological conditions (i.e. 37^0C, $-\log[H] = 7.4$, 5 mM HCO_3^-) are $10^{19.3}$ and $10^{20.3}$, ligands that have conditional binding constants with Ga(III) which are several orders of

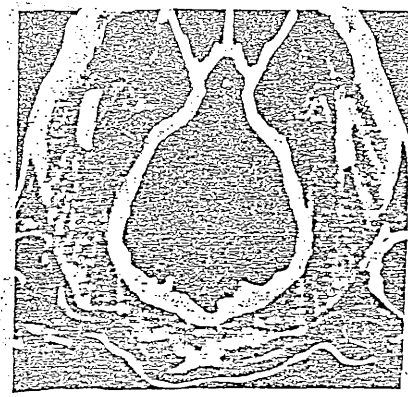


Figure 2. NMR scan of dog brain 27 hours after inoculation with Gd(III)-DTPA.[6]

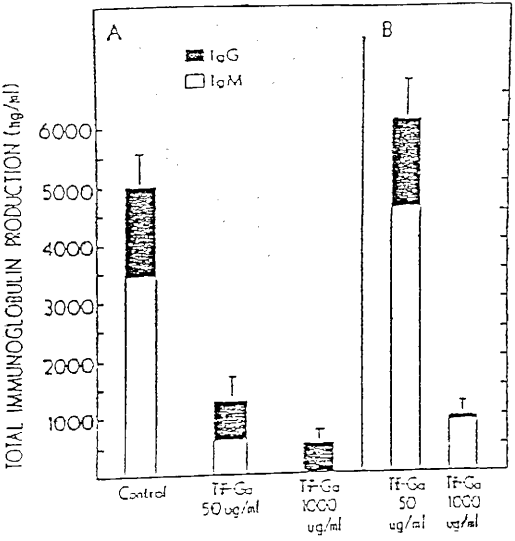


Figure 3. Effect of coordination of Ga(III) to transferrin (Tf-Ga) on Epstein Barr virus stimulated immunoglobulin production. Columns represent mean values and bars represent three standard deviations. A; Ga(III) added at the start of incubation. B; Ga(III) added after 4 days following the start of incubation.

magnitude greater than $10^{20.3}$ are required.[11] ([GaL*] is the sum of the concentrations of all of the forms of Ga(III) complexed with the ligand (transferrin); [Ga*] is the sum of the concentrations of all of the uncomplexed forms of Ga; and, [L*] is the sum of the concentrations of all of the uncomplexed forms of the ligand.) One reason for the strong affinity of transferrin for Ga(III) is that transferrin contains phenolate donor groups. Transferrin, at its active site, contains two phenolate oxygen donors (from tyrosine), two carboxylate oxygen donors (from glutamic acid), and two heterocyclic nitrogen donors (from histidine), plus a heterocyclic nitrogen (from histidine) hydrogen-bonded to bicarbonate (Figure 4).[12,13] In addition to a large affinity for Ga(III), In(III), Fe(III), and Gd(III) a rate of removal of these metal ions from transferrin which is rapid compared to various biological events is necessary. An example of such a biological event is glomerular filtration, which has been measured as 2.7×10^{-6} M sec^{-1} for Na(I).[14]

In addition to preventing coordination to Ga(III), In(III), Fe(III), and Gd(III) with transferrin, there is a need to prevent coordination of the metal ions to hydroxide in vivo. At 25°C and 0.1 M NaCl, $[Ga(OH)_4^-]/([Ga(III)][OH^-]^4) = 10^{39.0}$; therefore, at physiological conditions, including a -log[H] of 7.4, uncomplexed Ga(III) could be predominantly in the form of $Ga(OH)_4^-$.[15]

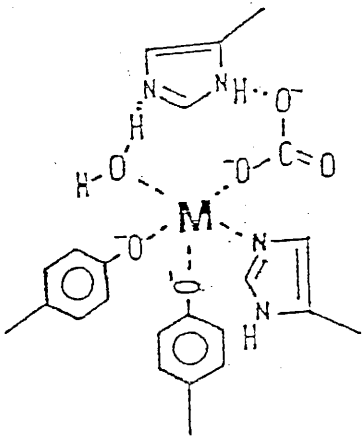


Figure 4. Proposed drawing of a metal to a binding site in transferrin.[13]

Similarly, large affinities of hydroxides for In(III) and for Fe(III) also exist (Table I).[16]

Design of Chelate Ligands

One of the most extensively studied chelate ligands is ehtylenediamine-N,N'-diacetic acid (EDTA). This ligand has a combination of protonation constants and a chelate stability constant for Ga(III) that gives a conditional stability constant of $10^{6.3}$ with Ga(III) at -log[H] = 7.4, 25°C, and I = 0.1 M.[17] Such an affinity for Ga(III) is insufficient to prevent complexation of Ga(III) with transferrin in vivo. EDTA has a smaller stability constant with Gd(III) and has slightly larger stability constants with In(III) and Fe(III).[17]

One way to improve the affinity of EDTA for the above metal ions is to replace some of the donor groups with hard (nonpolarizable) negative donor groups. The reason for using hard donor groups is that trivalent metal ions are hard Lewis acids and have high affinities for hard Lewis base donor groups.[18] Often the hardness of a donor group is indicated by its high pKa. Phenolate donors should then act as hard Lewis bases because the pKa of phenol is 10.0; therefore, HBED, a ligand similar in structure to EDTA with two carboxylate donor groups replaced by phenolate donor groups, should have significantly larger affinities for trivalent metal ions than EDTA (Table II).[19]

In regards to Fe(III) and Gd(III), another desirable

Table I. Hydrolysis Constants for Ga(III), In(III), and Fe(III).[16]

Equilibrium Quotient (K)	Log K
$[Ga(OH)]/([Ga][OH])$	10.9[a]
$[Ga(OH)_2]/([Ga][OH]^2)$	21.5[a]
$[Ga(OH)_3]/([Ga][OH]^3)$	30.9[a]
$[Ga(OH)_4]/([Ga][OH]^4)$	39.4[b]
$[In(OH)]/([In][OH])$	10.5[a]
$[In(OH)_2]/([In][OH]^2)$	20.3[a]
$[In(OH)_3]/([In][OH]^3)$	29.3[a]
$[In(OH)_4]/([In][OH]^4)$	33.9[b]
$[Fe(OH)]/([Fe][OH])$	11.17[a]
$[Fe(OH)_4]/([Fe][OH]^4)$	34.4[b]

[a] ionic strength (I) = 0.1 M., temperature (t) = 25^{0}C.

[b] ionic strength (I) = 0.0 M., temperature (t) = 25^{0}C.

Table II. Protonation Constants for EDTA (L) and Stability Constants for EDTA with Ga(III), In(III), Fe(III), and Gd(III). I = 0.1 M. t = 25°C.[17]

Equilibrium Quotient (K)	Log K
[HL]/([L][H])	10.19
$[H_2L]/([HL][H])$	6.13
$[H_3L]/([H_2L][H])$	2.69
$[H_4L]/([H_3L][H])$	2.0
[GaL]/([Ga][L])	21.0
[GaL]/([Ga(OH)L][H])	5.58
[InL]/([In][L])	24.9
[InL]/(In(OH)L][H])	8.49
[FeL]/([Fe][L])	25.1
[FeL]/([Fe(OH)L][H])	7.37
[GdL]/([Gd][L])	17.32

property for the chelate ligand is the presence of at least one site on the metal ion open for coordination with H_2O in the chelate complex. The reason an open site is desirable is due to the fact that paramagnetic effect, or the effect of the metal ion in relaxing the ^1H resonance of H_2O, is inversely proportional to the distance between the metal ion and the coordinated H_2O raised to the sixth power.[4] Thus, the H_2O should be in the inner coordination sphere of the metal ion. HBED is a six-coordinate ligand; however, open sites for H_2O coordination could still be available on Fe(III) and Gd(III) after coordination with HBED. Although Fe(III) usually has a coordination number of six, it can, as demonstrated by a crystal structure of a seven-coordinate Fe(III)(EDTA)(H_2O) complex, have a coordination number greater than six (Figure 5).[20] In addition, Gd(III) forms complexes with coordination numbers of eight or higher, as do most lanthanide compounds.[21]

Reasons for Equilibrium Measurements of HBED

There already have been some measurements made of the stability constants of HBED with various metal ions; however, there is some question as to the accuracy of the measured values. An early study reported the stability constants for HBED with the metal ions Co(II), Ni(II), Zn(II), Cu(II), and Fe(III); however, the constants were not determined for the metal ions Ga(III), In(III), or Gd(III).[22] Additionally, there is doubt as to the accuracy of this study's reported stability constant for HBED with

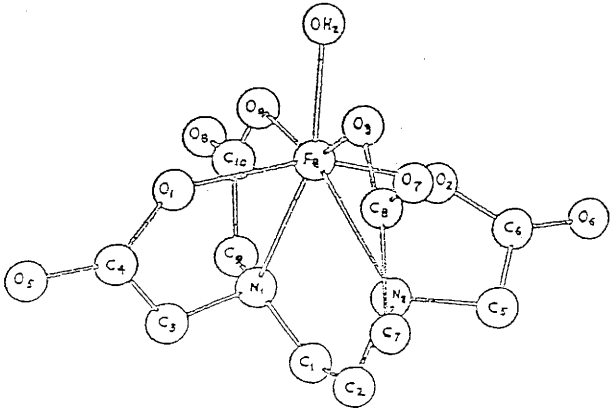


Figure 5. Skeleton model for Fe(III)(EDTA)(H_2O) complex.[20]

Fe(III). The authors determined the constant by an HBED-EDTA-Fe(III) spectrophotometric competition titration; therefore, any inaccuracy in the stability constant for EDTA with Fe(III) will produce an inaccuracy in the calculation of the stability constant for HBED with Fe(III). The value for the Fe(III)-EDTA stability constant used in the calculation of the Fe(III)-HBED stability constant was determined from an Fe(II)-Fe(III)-EDTA polarographic competition titration.[23] In turn, the value for the Fe(II)-EDTA stability constant used to calculate the Fe(III)-EDTA stability constant was determined from an Fe(II)-EDTA potentiometric titration.[24] Any error in either of these two studies would lead to an error in the calculated Fe(III)-HBED stability constant (Table III).

Another study does report the stability constants for HBED with In(III).[25] The procedure for determining this constant was not reported and the constant is believed to be incorrect. Because the constant is too large to have been determined from direct potentiometry (the constant is large enough so that there would be complete complexation even at the lowest -log[H] values of the titrations), some method besides direct potentiometry is necessary to determine the constant (Table IV).[25]

Also, a third study reports the stability constant of HBED with Ga(III). This constant was determined from a Ga(III)-Fe(III)-HBED spectrophotometric competition titration. Because the Fe(III)-HBED stability constant

Table III. Previously Reported Protonation Constants for HBED (L) and Stability Constants for HBED with Co(II), Ni(II), Zn(II), Cu(II), and Fe(III). I = 0.100 M (KNO_3). t = 25°C.[22]

Equilibrium Quotient (K)	Log K
[HL]/([L][H])	12.46
$[H_2L]/([HL][H])$	11.00
$[H_3L]/([H_2L][H])$	8.32
$[H_4L]/([H_3L][H])$	4.64
[CoL]/([Co][L])	19.89
[HCoL]/([CoL][H])	7.77
$[H_2CoL]/([HCoL][H])$	5.56
[NiL]/([Ni][L])	19.31
[HNiL]/([NiL][H])	8.51
$[H_2NiL]/([HNiL][H])$	6.45
[ZnL]/([Zn][L])	18.37
[HZnL]/([ZnL][H])	8.27
$[H_2ZnL]/([HZnL][H])$	5.99

Table III. Continued

Equilibrium Quotient (K)	Log K
[CuL]/([Cu][L])	21.38
[HCuL]/([CuL][H])	8.63
$[H_2CuL]/([HCuL][H])$	5.18
[FeL]/([Fe][L])	39.68

Table IV. Previously Reported Stability Constants for HBED (L) with Ga(III) and In(III).

Equilibrium Quotient (K)	Log K
[GaL]/([Ga][L])	39.57[a]
[InL]/([In][L])	39.66[b]

a Ref. 32, I= 0.100 M (KNO_3), t = 25.00°C.

b Ref. 25, I = 0.100 M (KCl), t = 25.00°C.

determined in the previously mentioned study was used to calculate the Ga(III)-HBED stability constant in this study, any error in the Fe(III)-HBED stability constant would lead to an error in the Ga(III)-HBED stability constant.[26]

Determining the stability constants for HBED with the metal ions Co(II), Ni(II), Cu(II), Ga(III), In(III), Fe(III), and Gd(III) would correct the inaccuracies for the previously measured stability constants of HBED with Ga(III), In(III), and Fe(III). In addition, the accuracy of the stability constants of HBED with the above divalent metal ions would be checked. Also, a previously undetermined stability constant, that of Gd(III) with HBED, will be determined. With reliable stability constants for HBED with the above metal ions correlations of the constants with factors such as the radii of the metal ions can be made. Also, correct values of metal ion activities (such as $-\log[M^{n+}]$, or pM) under various solution conditions can be carried out, and the values thus obtained may be compared with those of other ligands with the metal ions of interest.

EXPERIMENTAL

Preparation of Solutions

The solvent used for all of the solutions was boiled and cooled (CO_2 free) doubly distilled H_2O. With the exception of the standard concentrated HCl solution and the KOH solution, enough KCl was added to all of the solutions prepared for the ionic strength (I) to approximately equal 0.100 M.

The KOH solutions were prepared by diluting an ampule of VWR Scientific Dilut It KOH to approximately 1.0 L with the H_2O. The [KOH] was determined by titration against a standard potassium hydrogen phthalate solution to a phenolphthalein end point. To check for carbonate contamination of the KOH a Gran plot was performed. In a Gran plot, the KOH is titrated by a standard concentrated HCl solution. In the acid region, $(V + v)(10^{m.r.})$ is plotted versus v, giving v_e where the x axis is crossed; and, in the base region, $(V + v)(10^{m.r.})$ is plotted versus v, giving v_e' where the x axis is crossed. V is the initial volume of the solution; v is the volume of HCl added; and, m.r. is the pH meter reading. $v_e = v[HCl]/([KOH] + 2[(CO_3)^{2-}])$ and $v_e' = v[HCl]/[KOH]$; therefore, the difference between v_e and v_e' gives the $[(CO_3)^{2-}]/[KOH]$.[27] A least square FORTRAN computer program, LEAST, was use to determine the points where the x axis was crossed.[28]

The standard concentrated HCl solutions were prepared by diluting a stock HCl (36.5-38.0%) solution. The concentration of the HCl solutions were determined by titrations against the standard KOH solutions to a phenolphthalein endpoint. For calibration curves, a standard diluted HCl solution was prepared by diluting 10.00 mL. of the standard concentrated HCl solution to 1.0000 L.

Metal solutions of Co(II), Cu(II), Ni(II), Zn(II), Gd(III), and Fe(III) were prepared from the metal chloride salts. Some standard concentrated HCl solution was added to these solutions to prevent precipitation of the metal hydroxides. The metal ion concentrations of these solutions were determined by titration against standardized Na_2EDTA to a visual endpoint, using specified indicators and titration conditions. The [Na_2EDTA] itself was determined by titration against a standard $ZnCl_2$ solution that was prepared by dissolving an accurately weighed amount of Zinc metal in concentrated HCl.[29]

Ga(III) and In(III) solutions were prepared by dissolving accurately weighed amounts of the metal in concentrated HCl. Most of the HCl was allowed to evaporate over heat before the metal chloride solutions were diluted. The [HCl] in the Ga(III) and In(III) solutions were determined by titrating a 1:1 metal:Na_2EDTA solution by the standardized KOH to a phenolphthalein endpoint. The Na_2EDTA was added to prevent precipitation of the metal hydroxides.

HBED stock solutions were prepared from HBED crystals provided by Strem Chemicals. The [HBED] in the stock solutions and the millimoles of excess acid (from the HBED crystals) per millimoles of HBED in the stock solutions were determined from the HBED -log[H] profile. Inflection points exist at a (mmole of KOH per mmole of HBED) = 1.000 and at a = 2.000 for the plot of -log[H] versus a. The millimoles of KOH between a = 1.000 and a = 2.000 give the millimoles of HBED in the titration, and thereby the [HBED] in the stock solution. Additionally, the millimoles of KOH added at a = 1.000 minus the millimoles of HBED in the titration is equal to the millimoles of excess acid present per 1.000 millimole of HBED in the HBED stock solution.

Potentiometric Measurements

Potentiometric titrations were performed using a Corning pH meter 130 connected to a combination glass electrode and calomel reference electrode system. The titrations were conducted in a sealed titration vessel. The air in the titration vessel was expelled by passing through N_2 gas that had first been passed through a 1.0 M KOH solution and then a 0.1 M KCl solution. Likewise, the KOH was stored in a sealed bottle where the air had been expelled by the N_2 gas. Temperature for the titrations was kept at 25.00^{0}C by a Haake constant temperature bath.

The titrations were performed by adding measured amounts of the standard HBED and metal ion (if used) solutions, enough approximately 0.100 M KCl solution to

bring the volume of the titration vessel solution to about 50.00 mL., and enough of the standard KOH to give an initial a for the titration of about 5.000. For the titrations, the concentrations of HBED and of the metal ions were approximately 1.000×10^{-3} M. During the titrations increments of the standard concentrated HCl solution were added with a Gilmont microburet. The titrations were performed as back titrations with HCl rather direct titrations with the standard KOH because of the possibility of metal hydroxide precipitation. If the metal-HBED complexes were not stable enough to prevent precipitation of the metal hydroxides, this would be known at the beginning of the titration. For the titrations, the volume of HCl added was converted to the volume of KOH by the equation (volume of KOH added) = (volume of KOH initially added) - ([HCl]/[KOH])(volume of HCl added). Additionally, a slight excess of HBED to metal ion over a 1:1 ratio was used to prevent precipitation of metal hydroxides. A direct titration, where only the HBED solution was initially added and then increments of the standard KOH were added, was performed for the HBED alone.

Before each titration was performed, the pH meter system was calibrated through the use of a calibration curve. In a calibration curve, the standard KOH solution was titrated by the standard diluted HCl solution. At each point in the calibration, the -log[H] was calculated and the

pH meter reading was recorded. From this data, the values of a and b in the equation -log[H] = a(meter reading) + b were determined using the LEAST computer program. Then, during the following titrations, the pH meter readings were converted to -log[H] values through the calibration curve equation.

In order for the calibration curves preceding titrations to be performed, the value of $-\log K_w$ (-log[H][OH]) was determined form earlier titrations of the standard diluted HCl by the standard KOH. In these titrations, a calibration curve was calculated from the acid region alone and this curve was used to convert the pH meter readings in the base region to -log[H] values. At each point these values were multiplied by the -log[OH] values calculated form the stoichiometry of the titration to give $-\log K_w$. These $-\log K_w$ values were averaged for the titration. Four titrations were performed and their average $-\log K_w$ values were averaged to give a final $-\log K_w$ value of 13.824. This $-\log K_w$ value is in good agreement with the previously published $-\log K_w$ literature value of 13.78.[16]

The HBED protonation constants (with the exception of the most basic protonation constant) were calculated from the HBED titration curve using the FORTRAN computer program BEST.[30] This program determines, from the stoichiometric data, the equilibria necessary to calculate the -log[H] values that would correspond best to the observed -log[H]

values in the given titration. Similarly, the HBED-metal ion chelate formation, protonation, and hydroxy constants for Co(II), Ni(II), Zn(II) In(III), and Gd(III) were calculated from the HBED-metal ion titration curves using the BEST computer program.

Spectrophotometric Measurements

The most basic protonation constant for HBED, as well as the HBED-metal ion chelate formation constants for Cu(II), Fe(III), and Gd(III), were determined from UV-visible spectrophotometric titrations at varying -log[H]'s. spectrophotometric measurements were necessary for the most basic protonation constant of HBED because not enough of the totally deprotonated species of HBED (L) was present during any part of the potentiometric titration to calculate the most basic protonation constant. Likewise, not enough of the free metal species of Cu(II), Fe(III), or Ga(III) were present at any part of their respective potentiometric titrations to calculate their formation constants with HBED.

The spectrophotometric measurements were recorded using a Perkin-Elmer 553 Fast Scan UV/VIS Spectrophotometer. Concentrations used were approximately 1.000×10^{-4} M for HBED in the HBED spectrophotometric titration; 5.000×10^{-5} M for the Cu(II) and HBED in the Cu(II)-HBED titration; and, 2.000×10^{-4} M for the Fe(III) and HBED in the Fe(III)-HBED titration. In all instances, the spectrophotometric titrations were performed at 25.00^{0}C; and, cells with a 1.00 cm. path length were used.

For the HBED titration, at each -log[H] the spectrophotometer scanned from 400.0 nm. to 225.0 nm. in wavelength. The reason for this range is that the protonated phenolates in HBED have a maximum extinction coefficient at 277.5 nm. and the deprotonated phenolates have a maximum extinction coefficient at 292.5 nm. These absorbance correlate with earlier observations of phenolate absorbance at approximately 292 nm.[22] The logK value for the equilibrium quotient [HL]/([H][L]) was calculated from the absorbance at -log[H] values greater 10.000 using the FORTRAN computer program ABSPKAS.[31] This program determines, from the analytical concentration of the ligand and the -log[H] values, the equilibria and the extinction coefficients necessary to calculate the absorbance values that would correspond best to the observed absorbance values for a given spectrophotometric titration. The extinction coefficient for the totally deprotonated HBED species (ϵ_L) was determined from the absorbance at 292.5 nm. at an extreme alkaline condition (-log[H] of approximately 14.4). Because of the uncertainty of $-\log K_w$, and therefore -log[H], at this alkalinity, this absorbance was not used in the calculation of the most basic protonation constant of HBED. As a result of determining ϵ_L independently, the ABSPKAS program only needed to refine for the extinction coefficients for the mono- and di-protonated HBED species (ϵ_{HL} and ϵ_{H_2L}) in addition to the most basic protonation

constant of HBED. Because the addition of KOH diluted the titration vessel solution, the measured absorbances were converted to absorbance corrected values by the equation (absorbance corrected) = ((absorbance measured)(volume measured)/(initial volume)).

For -log[H] values above 11.824 a significant portion of the ionic strength comes from KOH. As a result, the potentiometric calibration curve deviates from linearity and measured -log[H] values are unreliable. For these measurements, the -log[H] values were calculated directly from the amount of standard KOH added, as the effect on -log[H] from the HBED equilibria is negligible in this region of alkalinity. In addition, for these measurements a 25.00 mL. solution was made in a volumetric flask for each absorbance reading.

For the spectrophotometric determination of the formation constants of HBED with Cu(II), Fe(III), and Ga(III) the measurements were performed on 25.00 mL. solutions prepared for each scan. The -log[H] values for the scan solutions were calculated from the amount of standard concentrated HCl added. The -log[H] values ranged from 2.500 to approximately 1.000.

In the spectrophotometric determination of the Cu(II)-HBED formation constant the spectrophotometer scanned from 440.0 nm. to 225.0 nm.; and, the constant was calculated from the absorbance at 277.5 nm. At 277.5 nm., the

absorbing species are H_4L, H_5L, and H_2CuL (the diprotonated chelate complex). The $\log K_{ML}$ (the formation constant between Cu(II) and the completely deprotonated form of HBED) is calculated form the equation $K_{H_2CuL}/K_H^4 = [H_2CuL][H]^2/([Cu(II)][H_4L])$. $K_H^4 = [H_4L]/([H]^4[L])$. This value is determined from the potentiometric titration of HBED alone. [H] is measured from the pH meter or calculated from the amount of HCl added to the 25.00 mL. solution. $[Cu(II)] = T_{Cu} - [H_2ML]$, where T_{Cu} is the total concentration of Cu in the solution; and, $[H_4L] = T_L - [H_2CuL] - [H_5L]$, where T_L is the total concentration of all species of HBED in the solution.

In order to determine $[H_2CuL]$, the equation (absorbance) $= e_{H_4L}[H_4L] + e_{H_5L}[H_5L] + e_{H_2CuL}[H_2CuL]$ was used. The extinction coefficients for the diprotonated chelate complex (e_{H_2CuL}) was determined from the absorbance of a solution containing Cu(II) and HBED at a high enough -log[H] where H_2CuL is the only absorbing species.

The log values of the two chelate protonation constants (determined from the potentiometric titration of Cu(II) and HBED) were subtracted from the calculated value for K_{H_2CuL} to give $\log K_{ML}$. Because HCl has a greater effective ionic strength than KCl, large concentrations of HCl (low -log[H]'s) changed the effective ionic strength of the solutions. To account for this, the LEAST computer program calculated the slope for $\log K_{ML}$ versus [HCl]/([HCl] +

[KCl]). The y intercept ([HCl]/([HCl] + [KCl]) = 0.00) gives the corrected $\log K_{ML}$ value.

To calculate the Fe(III)-HBED formation constant the spectrophotometer scanned from 600.0 nm. to 225.0 nm. and the constant was calculated from absorbances measured at 470.0 nm. 470.0 nm. was used because at this wavelength the Fe(III)-HBED complex and the monoprotonated complex absorb while neither the uncomplexed Fe(III) nor the uncomplexed HBED absorb. The absorbances in the -log[H] range of 2.000 to 1.650 were used to calculate the extinction coefficients for both the FeL and HFeL species (the equilibrium constant for the protonation of the complex was determined from the potentiometric titration of Fe(III) and HBED). The absorbances in the -log[H] range of 1.500 to 1.180 were used to calculate the $\log K_{ML}$ value for Fe(III) and HBED.

The equation used for the calculation was $K_{ML} = (([H]^5[FeL])/([H_5L][Fe(III)]))(K_5^H)$. $K_5^H = [H_5L]/([H]^5[L])$ and is determined from the potentiometric titration of HBED without any metal ion. [H] is calculated from the amount of HCl added to the 25.00 mL. solutions; [FeL] is determined from the absorbances; $[H_5L] = T_L - [FeL] - [HFeL]$; and, $[Fe(III)] = T_{Fe} - [FeL] - [HFeL]$. As with Cu(II), the slope of calculated $\log K_{ML}$ values for Fe(III) and HBED versus [HCl]/([HCl] + [KCl]) was calculated; and, the y intercept gave the corrected $\log K_{ML}$ value.

The Ga(III)-HBED formation constant was determined from the reduction in the absorbances at 470.0 nm. of a 1:1 (approximately 2.000×10^{-4} M) Fe(III):HBED solution from the addition of Ga(III). Because neither the uncomplexed Ga(III) nor the Ga(III)-HBED complex, as well as the uncomplexed Fe(III) and the uncomplexed HBED, absorb at this wavelength, the reductions in the absorbances are measurements of the degree to which Ga(III) outcompetes Fe(III) for complexation with HBED. Two different concentrations of Ga(III) were used to calculate the Ga(III)-HBED formation constant.

RESULTS

Protonation Constants for HBED

The potentiometric titration curve for HBED contains inflection areas at values of (mmol KOH)/(mmol HBED) (a) = 1.000 and 2.000 (Figure 6). At a > 2.00, the phenolate oxygens begin to deprotonate. The -log[H] values in this region are too high to determine the protonation constants by potentiometry. Below a = 2.00 there are three protonation constants that were determined from the BEST calculation of the titration curve (Table V). These values correspond to protonation of the amine nitrogens and protonation of one carboxylate oxygen on HBED. The protonation of the second carboxylate oxygen does not occur in any measurable amount during the titration, thus behaving as a strong acid at least for a solution of -log[H] > 2.000.

The protonation constants for the two phenolate oxygens were determined from spectrophotometric titration. The titration is shown in Figures 7 and 8. At a wavelength of 292.5 nm. the protonated phenolates have their maximum absorbances, while at 277.5 nm. the deprotonated phenolates have their maximum absorbances. It is from the data at the 277.5 nm. wavelength that the protonation constants for the phenolates were determined. The protonation constants are listed in Table V. The extinction coefficients calculated

Figure 6: Potentiometric titrations of HBED with divalent metal ions. I = 0.100 M (KCl). t = 25.00^{0}C. a, 1.689 x 10^{-3} M HBED; b, 6.719 x 10^{-4} M Zn(II), 6.845 x 10^{-4} M HBED; c, 1.012 x 10^{-3} M Ni(II), 1.022 x 10^{-3} M HBED; d, 1.028 x 10^{-3} M Co(II), 1.044 x 10^{-3} M HBED; e, 9.492 x 10^{-4} M Cu(II), 9.738 x 10^{-4} M HBED.

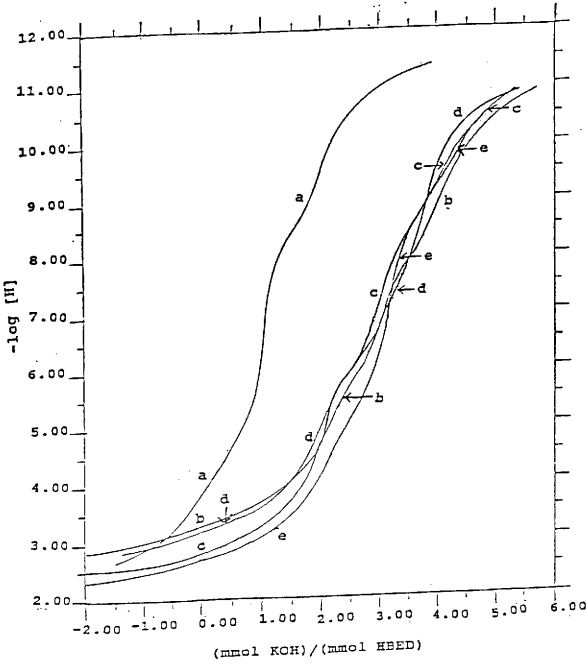

12.00
11.00
10.00
9.00
8.00
7.00
6.00
5.00
4.00
3.00
2.00
-log [H]
-2.00
-1.00
0.00
1.00
2.00
3.00
4.00
5.00
6.00
(mmol KOH)/(mmol HBED)
a
b
c
d
e

Table V. Stability Constants for Divalent Metal Ions with HBED. I = 0.100 M (KCl). t = 25.00°C.

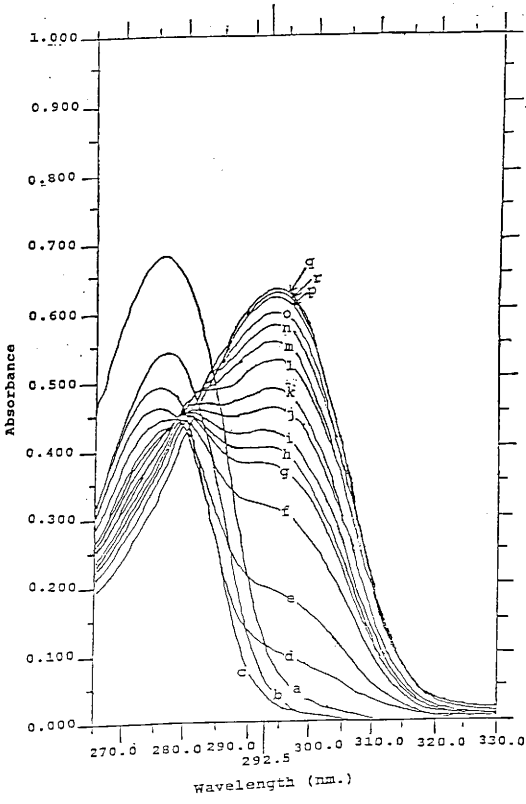
Equilibrium Quotient (K)	Log K		
	a	b	c
$[HL]/([L][H])$	12.35	12.46	12.60
$[H_2L]/([HL][H])$	11.08	11.00	11.00
$[H_3L]/([H_2L][H])$	8.45	8.32	8.44
$[H_4L]/([H_3L][H])$	4.76	4.64	4.72
$[H_5L]/([H_4L][H])$	2.18	----	2.57
$[ZnL]/([Zn][L])$	19.11	18.37	----
$[HZnL]/([ZnL][H])$	7.91	8.27	----
$[H_2ZnL]/([HZnL][H])$	5.79	5.99	----
$[CoL]/([Co][L])$	19.18	19.89	----
$[HCoL]/([CoL][H])$	7.93	7.77	----
$[H_2CoL]/([HCoL][H])$	5.97	5.46	----
$[NiL]/([Ni][L])$	19.99	19.31	----
$[HNiL]/([NiL][H])$	8.54	8.51	----
$[H_2NiL]/([HNiL][H])$	6.65	6.45	----

Table V. Continued.

Equilibrium Quotient (K)	Log K		
	a	b	c
[CuL]/([Cu][L]	23.40	21.38	23.69
[HCuL]/([CuL][H])	8.34	8.63	8.49
$[H_2CuL]/([HCuL][H])$	4.66	5.18	5.04

[a]This work. [b]Ref. 22. [c]Ref. 25.

Figure 7. Spectrophotometric titration of HBED at varying -log [H]'s. $[HBED]_{initial}$ = 1.235 x 10^{-4} M. I = 0.100 M (KCl). t = 25.00^{0}C. The -log [H] values are: a = 3.937; b = 4.336; c = 6.126; d = 8.735; e = 9.496; f = 10.117; g = 10.387; h = 10.491; i = 10.618; j = 10.791; k = 10.926; l = 11.064; m = 11.242; n = 11.395; o = 11.521; p = 11.669; q = 11.827; and, r = 11.980.


1.000
0.900
0.800
0.700
0.600
0.500
0.400
0.300
0.200
0.100
0.000
Absorbance
270.0
280.0
290.0
292.5
300.0
310.0
320.0
330.0
Wavelength (nm.)
a
b
c
d
e
f
g
h
i
j
k
l
m
n
o
p
q

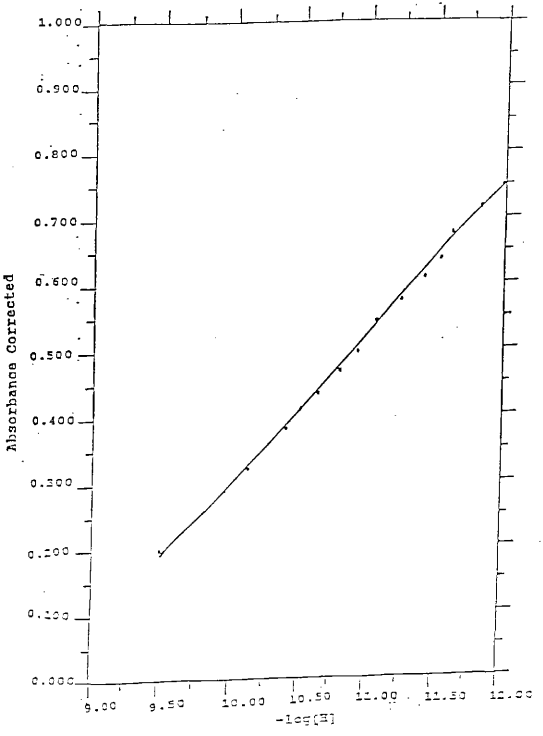


Figure 8. Plot of HBED spectrophotometric titration for -log [H] > 9.000 (wavelength = 277.5 nm.).

from the absorbances are: $\epsilon_L = 7.450 \times 10^3\ M^{-1}$, $\epsilon_{HL} = 5.346 \times 10^3\ M^{-1}$, and $\epsilon_{H_2L} = 2.194 \times 10^3\ M^{-1}$.

Stability Constants for HBED with Divalent Metal Ions

The potentiometric titration curves for HBED with divalent metal ions are shown in Figure 6. The titrations of HBED with the metals Zn(II), Co(II), Ni(II), and Cu(II) show small inflection regions near a = 2.00, 3.00, and 4.00. These inflection areas occur slightly before the integral a values as a result of the titration solutions containing a slight excess of HBED in relation to the metal ions. What produces the three inflection areas is the formation of protonated metal chelate complexes in addition to unpotonated chelate complexes of the divalent metal ions. Each of the four divalent metal chelate complexes has two protonation constants, one which is slightly basic and one which is slightly acidic (Table V). Although the structures of the diprotonated and mono- protonated chelate species are not known, the protonated chelate species likely have protonated phenolates which are not coordinated to the metal ions. Such structures are likely because of the large affinity the phenolates on HBED have for protons. Spectrophotometric measurements at -log[H] values near the complex protonation constants would indicate if the protonated complexes contain protonated phenolates. The absorbances around 277.5 nm. and 292.5 nm. would change in the -log[H] regions corresponding to the complex protonation constants.

The calculated stability constants for the divalent metal ions with totally deprotonated HBED are listed in Table V. The stability constants for Zn(II), Co(II), and Ni(II) were satisfactorily determined from the potentiometric data. That is, in the low -log[H] region of the titration curves there were sufficiently large (>20%) proportions of the metal ions and HBED uncomplexed with each other. An example of this is the species distribution shown for Co(II) with HBED in Figure 9. A species distribution shows the relative amounts of the various species present in the solution as a function of the -log[H] of the solution. In this figure, the % species = ([species]/$[HBED]_{total}$) x 100. For the Co(II)-HBED titration, the % species for uncomplexed Co(II) reaches 100 % at -log[H] values greater than the lowest -log[H] values of the titration. Likewise, the combined % species for the uncomplexed species of HBED, H_4L and H_5L, reach 100 % at the same -log[H] values.

Conversely, the potentiometric titration of Cu(II) with HBED was insufficient to calculate the stability constant for Cu(II) with HBED. The spectrophotometric titration of Cu(II) with HBED at varying low -log[H]'s was sufficient to determine the stability constant (Figure 10 and Figure 11). The measurements were made at 277.5 nm., the wavelength at which the protonated phenolates absorb. Apparently, the coordination of Cu(II) has a similar but more pronounced effect on the phenolates that protonation does. The

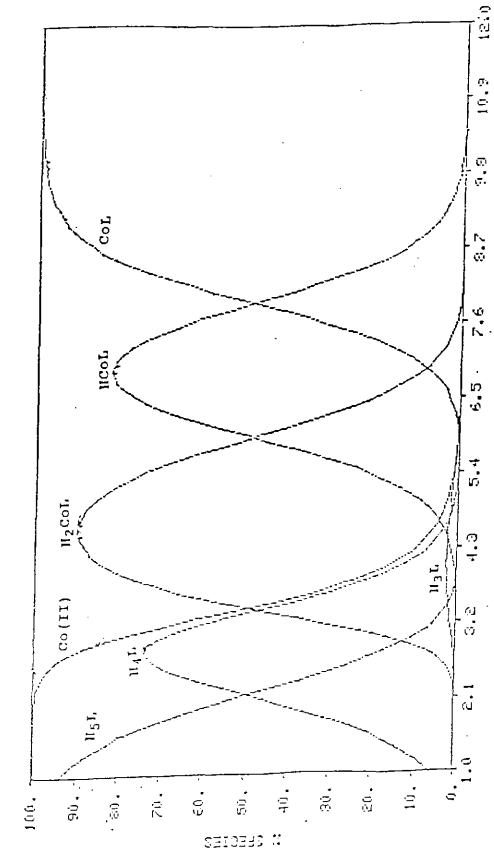
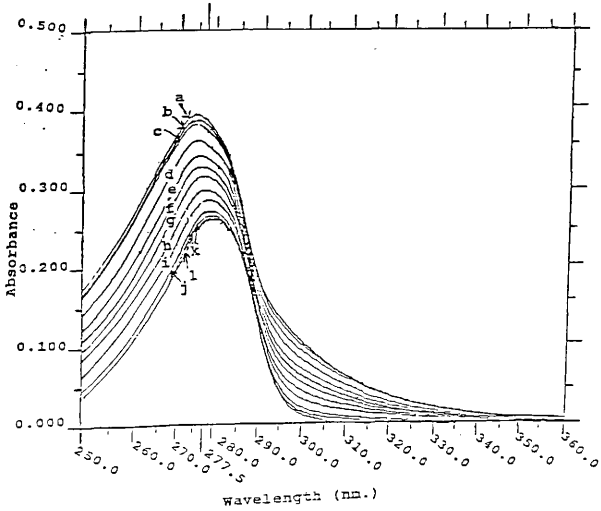


Figure 9. Species distribution for Co(II) and HMED.

Figure 10. Spectrophotometric titration of Cu(II) and HBED at varying -log [H]'s. [HBED] = 3.942×10^{-5} M. [Cu(II)] = 4.868×10^{-5} M. I = 0.100 M (KCl). t = 25.00^{0}C. The -log [H] values are: a = 3.606; b = 2.516; c = 2.223; d = 2.154; e = 2.018; f = 1.946; g = 1.856; h = 1.760; i = 1.663; j = 1.557; k = 1.439; and, l = 1.346.


0.500
0.400
0.300
0.200
0.100
0.000
Absorbance
a
b
c
d
e
f
g
h
i
j
k
l
250.0
260.0
270.0
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280.0
290.0
300.0
310.0
320.0
330.0
340.0
350.0
360.0
Wavelength (nm.)

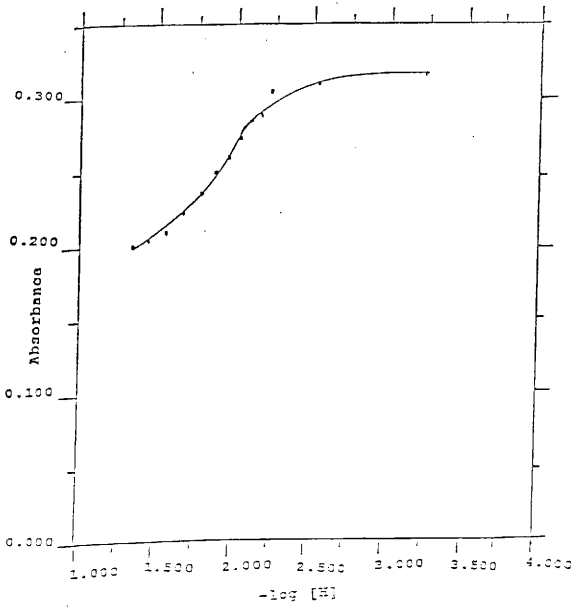


Figure 11. Plot of Cu(II)-HBED spectrophotometric titration.

calculated extinction coefficients are: ϵ_{H_2CuL} = 1.016 x 10^4 M^{-1}, ϵ_{H_4L} = 2.098 x 10^3 M^{-1}, and ϵ_{H_5L} = 2.098 x 10^3 M^{-1}. As the spectrophotometric measurements went to lower -log[H] values (the [HCl]/([HCl] + [KCl]) increased) the calculated $\log K_{CuL}$ values between Cu(II) and HBED became smaller (Table VI). This can be explained from considering the charges of the species in solution. The predominant reactions occurring at these -log[H] values are $H_5L^+ + Cu^{2+} = H_2CuL^0 + 3H^+$ and $H_4L^0 + Cu^{2+} = H_2CuL^0 + 2H^+$. Here, a +2 charged metal is reacting to form an uncharged chelate species. An increase in effective ionic strength would be a deterrent for such a reaction. The species distribution for Cu(II) with HBED shows that the % species of Cu(II) reaches nearly 100 % during the spectrophotometric titration (Figure 12). Actually, the conditions are even slightly more favorable than shown. The species distribution was calculated from the stability constant given in Table V. At the lower -log[H] values, the stability constant decreases slightly and the % species of Cu(II) increases slightly.

Stability Constants for HBED with Trivalent Metal Ions

The potentiometric titration curves for HBED with trivalent metal ions are shown in Figure 13. In contrast to the divalent metal ions the potentiometric titration curves for the trivalent metal ions only show inflection regions at values near a = 4.00. Again, because a slight excess of HBED in relation to the metal ions was used, the inflection

Table VI. Values of Log $K_{Cu(II)L}$ for Varying HCl Mole Fractions of Solution Ionic Strength.

[HCl]/([HCl] + [KCl]) (x)	Log $K_{Cu(II)L}$ (y)
0.2773	22.285
0.2173	22.950
0.1738	23.109
0.1393	23.111
0.1132	23.123
0.09594	23.223

y intercept: Log $K_{Cu(II)L}$ = 23.40

slope: -2.03 (Log $K_{Cu(II)L}$)([HCl] + [KCl])/[HCl]

correlation coefficient: -0.97

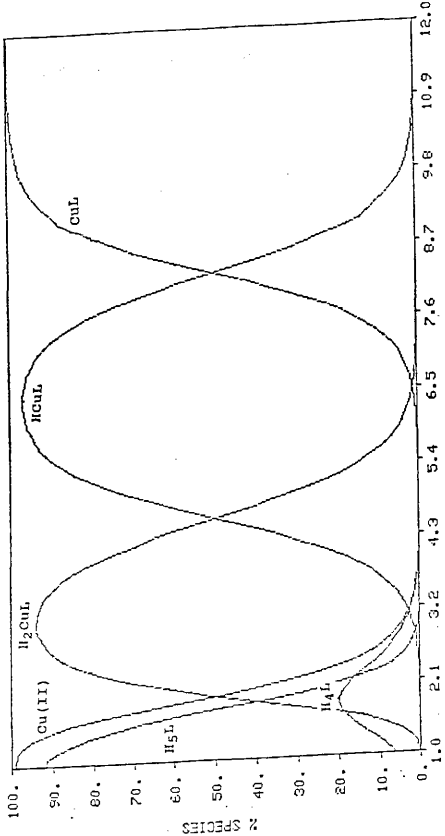
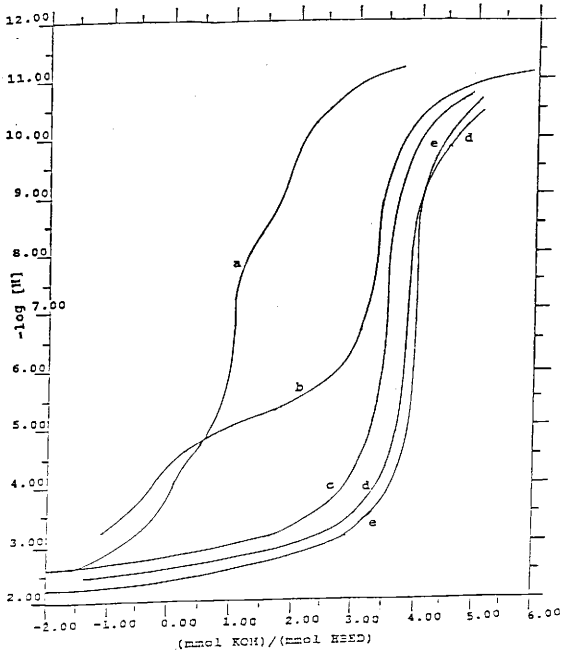


Figure 12. Species Distribution for Cu(II) and HBED.

Figure 13. Potentiometric titrations of HBED with trivalent metal ions. I = 0.100 M (KCl). t = 25.00^{0}C. a, 1.689 x 10^{-3} M HBED; b, 8.367 x 10^{-4} M Gd(III), 8.908 x 10^{-4} M HBED; c, 8.152 x 10^{-4} M In(III), 9.262 x 10^{-4} M HBED; d, 6.579 x 10^{-4} M Ga(III), 6.671 x 10^{-4} M HBED; e, 1.099 x 10^{-3} M Fe(III), 1.105 x 10^{-3} M HBED.


12.00
11.00
10.00
9.00
8.00
7.00
6.00
5.00
4.00
3.00
2.00
-log [H]
-2.00
-1.00
0.00
1.00
2.00
3.00
4.00
5.00
6.00
(mmol KOH)/(mmol HEED)
a
b
c
d
e
e
d

areas occur at a values slightly less than 4.00. The lack of any other inflection areas in the titration curves is a result of chelate protonation constants which are either small (fairly acidic) or not present (Table VII).

The calculated stability constants for Gd(III), In(III), Fe(III), and Ga(III) with totally deprotonated HBED are given in Table VII. The potentiometric titrations themselves were sufficient to determine the stability constants of Gd(III) and In(III) with HBED. An example of this is shown by the species distribution of Gd(III) with HBED (Figure 14). Here, as with Co(II) (Figure 9), the % species of uncomplexed Gd (Gd(III)) and HBED (H_3L + H_4L + H_5L) reach 100 % within the -log[H] range of the titration. The primary difference between the species distributions for Gd(III) and Co(II) is that there is much less presence of the diprotonated and monoprotonated chelate species for Gd(III).

The potentiometric titrations were insufficient to determine the stability constants for Fe(III) and Ga(III) with HBED. As with Cu(II), a spectrophotometric titration of Fe(III) and HBED at varying low -log[H]'s was sufficient to determine the stability constant of Fe(III) with HBED. The spectrophotometric titration is shown in Figure 15 and Figure 16. The calculation were made at 470.0 nm. As shown in Figure 16, there is little change in absorbance per change in -log[H] in the -log[H] range 2.00 to 1.65. It is in this region that the extinction coefficients for FeL and

Table VII. Stability Constants for Trivalent Metal Ions with HBED. I = 0.100 M (KCl). t = 25.00°C.

Equilibrium Quotient (K)	Log K			
	a	b	c	d
[HL]/([L][H])	12.35	12.46	12.60	----
[H_2L]/([HL][H])	11.08	11.00	11.00	----
[H_3L]/([H_2L][H])	8.45	8.32	8.44	----
[H_4L]/([H_3L][H])	4.76	4.64	4.72	----
[H_5L]/([H_4L][H])	2.18	----	2.57	----
[GdL]/([Gd][L])	18.79	----	----	----
[HGdL]/([GdL][H])	6.06	----	----	----
[H_2GdL]/([HGdL][H])	4.55	----	----	----
[InL]/([In][L])	27.90	----	39.66	----
[HInL]/([InL][H])	4.19	----	----	----
[FeL]/([Fe][L])	36.74	39.68	----	----
[HFeL]/([FeL][H])	2.16	----	----	----
[GaL]/([Ga][L])	37.73	----	----	39.57

[a]This work. [b]Ref 22. [c]Ref 25. [d]Ref. 32.

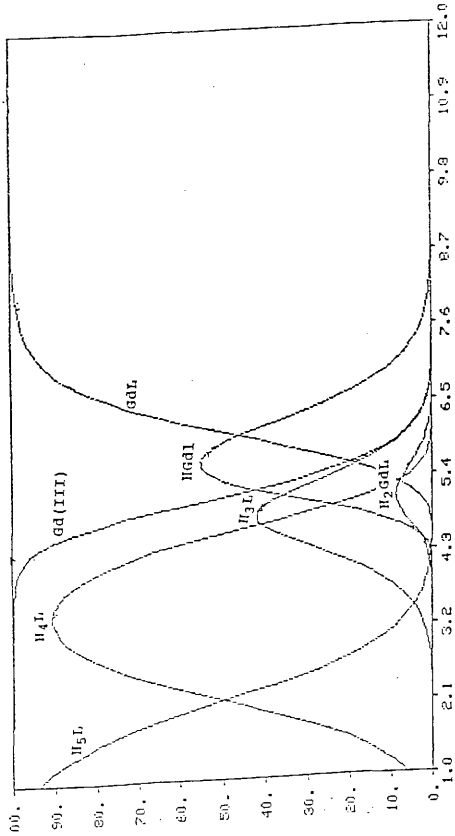
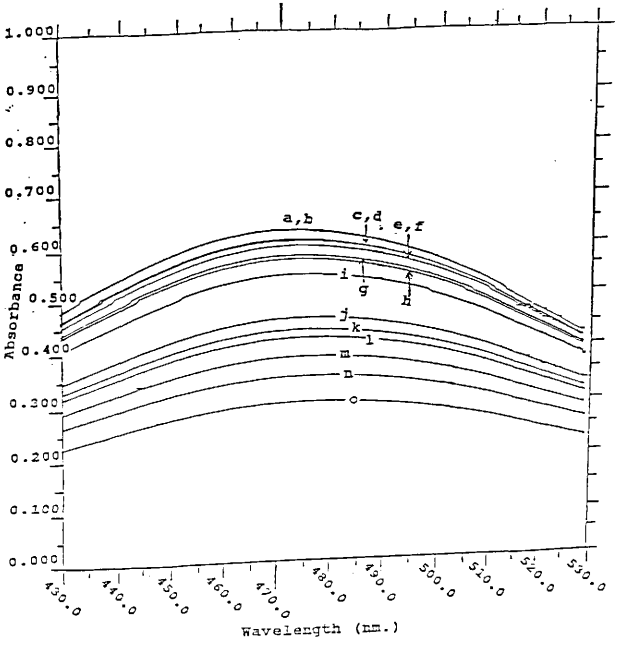


Figure 14. Species distribution for Gd(III) and HBED.

Figure 15. Spectrophotometric titration of Fe(III) and HBED at varying -log [H]'s. [HBED] = 2.144×10^{-5} M. [Fe(III)] = 2.088×10^{-5} M. I = 0.100 M (KCl). t = 25.00°C. The -log [H] values are: a = 2.008; b = 1.955; c = 1.791; d = 1.738; e = 1.690; f = 1.647; g = 1.608; h = 1.565; i = 1.480; j = 1.338; k = 1.319; l = 1.300; m = 1.265; n = 1.226; and, o = 1.187.


1.000
0.900
0.800
0.700
0.600
0.500
0.400
0.300
0.200
0.100
0.000
Absorbance
a,b
c,d
e,f
g
h
i
j
k
l
m
n
o
430.0
440.0
450.0
460.0
470.0
480.0
490.0
500.0
510.0
520.0
530.0
Wavelength (nm.)

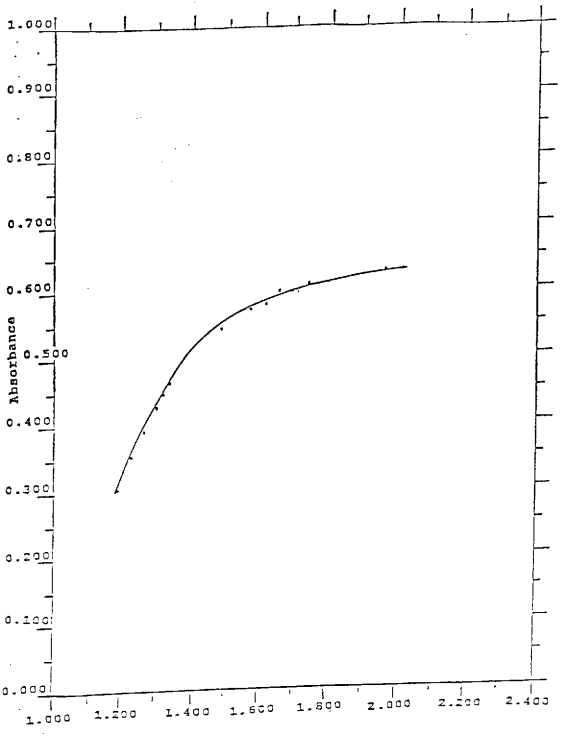


Figure 16. Plot of Fe(III)-HBED spectrophotometric titration.

HFeL were calculated. The calculated extinction coefficients are: $\epsilon_{FeL} = 3.248 \times 10^3\ M^{-1}$, and $\epsilon_{HFeL} = 2.796 \times 10^3\ M^{-1}$. Because there is little change in the extinction coefficient upon protonation of the chelate complex, a phenolic oxygen is probably not being protonated.

In the -log[H] region 1.50 to 1.20 there is much more of a change in absorbance per change in -log[H]. It is in this region that the stability constant of Fe(III) with HBED was calculated. As with Cu(II), with decreasing -log[H] (increasing [HCl]/([HCl] + [KCl]) the calculated $\log K_{FeL}$ values for Fe(III) with HBED decrease (Table VIII). Here, the predominant reactions are $H_5L^+ + Fe^{3+} = FeL^{1-} + 5H^+$ and $H_5L^+ + Fe^{3+} = HFeL^0 + 4H^+$. A 3+ charged metal ion is being coordinated into either an uncharged or a 1- charged chelate species. Perhaps because a 1- charged chelate species is produced in addition to an uncharged species, the variance of calculated $\mathrm{Log}K_{ML}$ values for Fe(III) with HBED is not as great for Cu(II) with HBED (the magnitude of the Fe(III) slope is less).

The calculated stability constant is listed in Table VII. The species distribution for Fe(III) with HBED shows that the % species for uncomplexed Fe (Fe(III)) and HBED (H_5L) reach about 20 % in the spectrophotometric titration (Figure 17). Actually, somewhat more uncomplexed Fe and HBED are produced because of the lowered stability constants in the low -log[H] region.

Table VIII. Values of Log $K_{Fe(III)L}$ for Varying HCl Mole Fractions of Solution Ionic Strength.

[HCl]/([HCl] + [KCl]) (x)	Log $K_{Fe(III)L}$ (y)
0.6501	35.91
0.5943	35.95
0.5437	36.00
0.5012	36.05
0.4797	36.07
0.4592	36.13
0.3311	36.33

y intercept: Log $K_{Fe(III)L}$ = 36.74

slope: -1.33 (Log $K_{Fe(III)L}$)([HCl] + [KCl])/[HCl]

correlation coefficient: -0.99

recalculated Log $K_{Fe(III)L}$ from ref. 22 = 40.75

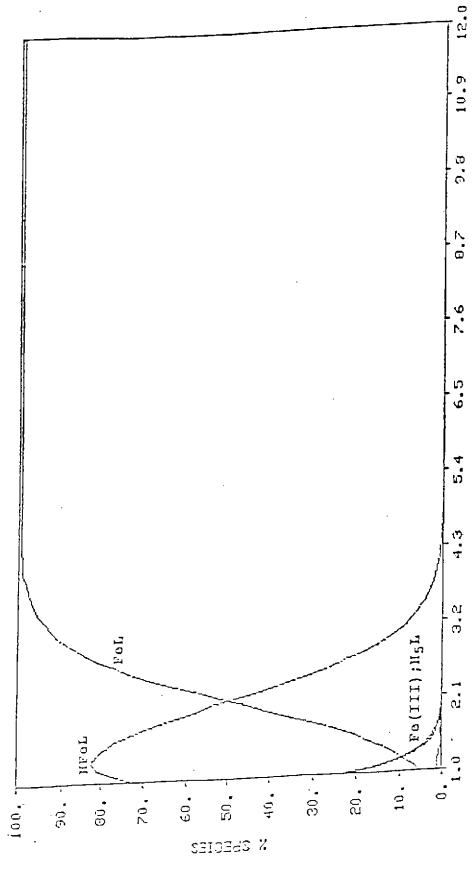


Figure 17. Species distribution for Fe(III) and HBED.

Once the Fe(III)-HBED stability constant was calculated, then the stability constant of Ga(III) with HBED was calculated from Ga(III)-Fe(III)-HBED spectrophotometric competition. From several solutions containing varying concentrations of Ga(III) and the same concentrations of Fe(III) and HBED, the calculations were made from two solutions where the concentrations of Ga(III) were such that the absorbance at 470.0 nm. was reduced somewhat (a measurable amount of Ga(III) had displaced Fe(III) from coordination with HBED) but not reduced to zero (a measurable amount of Fe(III) was left complexed with HBED). The results from these two solutions are given in Table IX.

Table IX. Spectrophotometric Data from Ga(III)-Fe(III)-HBED Competition. [HBED] = 1.563×10^{-4} M. [Fe(III)] = 1.729×10^{-4} M. I = 0.100 M (KCl). t = 25.00^{0}C.

-Log [H]	[Ga(III)]	Absorbance	Log $K_{Ga(III)L}$
2.715	1.136×10^{-4} M	0.206	37.67
2.554	2.271×10^{-4} M	0.080	37.78

average Log $K_{Ga(III)L}$ = 37.73

DISCUSSION

Comparison of Stability Constants with Previous Work

This work determined protonation constants for HBED, stability constants for HBED with Zn(II), Co(II), Ni(II), and Cu(II), and metal chelate protonation constants for HBED with the above metals that are in good agreement with two previous works.[22,25] Where there is major disagreement is in the stability constants for HBED with trivalent metal ions. There is no previously reported stability constant for HBED with Gd(III); however, the previously reported stability constants for HBED with In(III), Fe(III), and Ga(III) vary from two to twelve orders of magnitude (Table VII).[22,25,32] The greatest difference involves the stability constants for HBED with In(III). The previously reported constant for HBED with In(III) is twelve orders of magnitude greater than what is determined in this work; however, because no mention was made of how the constant was measured, there is no way to determine why the two reported values differ so much.[25]

There are several reasons why the previously determined value for the stability constant of HBED with Fe(III) is two orders of magnitude higher. The study calculated the constant from an Fe(III)-HBED-EDTA spectrophotometric competition titration. The values were calculated without consideration for the $[H_5L]/([H]^5[L])$ constant for either

HBED or EDTA; the values were calculated without consideration for the Fe(III)-EDTA chelate complex protonation constant that was determined in this work; and, the values were calculated assuming the literature value given for the stability constant between Fe(III) and EDTA is accurate.[22] From the spectrophotometric data the earlier study gave, the Fe(III)-HBED stability constant was recalculated including the previously ignored constants. Additionally, the constant was recalculated using a newly determined constant for Fe(II)-EDTA.[33] This recalculated value is listed in Table VIII. The difference between this value and the value determined in this work is even greater than the difference between the originally calculated value and this work. One reason may be the Fe(III)-EDTA constant, which was determined by an Fe(II)-Fe(III)-EDTA redox competition titration. The earlier Fe(II)-EDTA titration reported a smaller stability constant and a larger chelate complex protonation constant than the latter titration, both of which values were determined in the acid region of the titrations.[24,33] Because the calculations for the Fe(II)-Fe(III)-EDTA redox competition were made in a -log[H] range of 5.6 to 6.6, a reason for error in the Fe(II)-EDTA titration (e.g. oxygen contamination of the solution) could be a reason for error in the Fe(II)-Fe(III)-EDTA redox competition titration.[23]

There is also a two log unit disagreement between the stability constant for HBED with Ga(III) determined in this work and that in an earlier report.[32] That study also determined the Ga(III)-HBED stability from Ga(III)-Fe(III)-HBED spectrophotometric competition titration; however, the study calculated the Ga(III)-HBED stability constant from the Fe(III)-HBED stability constant that is two orders of magnitude higher than the one calculated in this work.[32]

Trends of Reported Stability Constants

For the divalent metal ions, the magnitudes of the stability constants with HBED follow the common trends of Co(II) < Ni(II) < Cu(II) and Zn(II) < Cu(II) (Table V). The stability constants for the trivalent metal ions were all much greater with HBED, with the exception of Gd(III). The magnitudes of the stability constants of the trivalent metal ions with HBED followed the trend Gd(III) < In(III) < Fe(III) < Ga(III). In fact, the LEAST calculation for the log values of the trivalent metal ion stability constants with HBED versus the reported ionic radii of the metal ions is highly linear with a large slope (Table X).[28,34] The preference of HBED for trivalent (hard) metal ions and the pronounced variance of $\log K_{ML}$ values as a result of the ionic radii of trivalent metal ions indicates that HBED favors bonding with metal ions that form chelate complexes that are largely ionic in character.

Also, the presence of chelate protonation constants indicates that not all the donor groups of HBED readily

Table X. List of Ionic Radii of Trivalent Metal Ions and Their Corresponding Stability Constants with HBED. I = 0.100 M (KCl). t = 25.00°C.

Metal Ion	Ionic radius(A)[a] (x)	Log K_{ML} (y)
Gd(III)	1.053	18.79
In(III)	0.800	27.90
Fe(III)	0.645	36.74
Ga(III)	0.620	37.73

y intercept: Log K_{ML} = 67.74

slope: -0.44 Log K_{ML}/A

correlation coefficient: -0.99

[a]Ref. 34.

coordinate with metal ions. What are the groups that do not coordinate is still unknown; however, there is reason to believe that the protonated chelate complex of Fe(III) and HBED has the caboxylate oxygen protonated. The HFeL species has an extinction coefficient at 470.0 nm. that is nearly as large as the extinction coefficient for the FeL species. If the phenolate oxygen was protonated, then the differences in extinction coefficients should be greater. Whether or not the second carboxylate oxygen is coordinated to Fe(III) or not is debatable because that carboxylate is totally deprotonated whether or not HBED is coordinated to a metal ion. Figure 18 shows a proposed structure for the HFeL species that shows neither carboxylate oxygen coordinated to Fe(III).

Suggestions for Further Studies

In addition to divalent and trivalent metal ions, the determination of the stability constants of HBED with tetravalent metal ions (such as Th(IV) and Ge(IV)) should be of interest, considering the high affinity HBED has for hard metal ions. In addition, with the large impact that the ionic radius of the metal ion has on its stability constant with HBED, measurements of trivalent metal ions with ionic radii outside of the bounds confined to this work would yield additional useful data. The constants from metals with smaller ionic radii than those measured (e.g. Al(III)) would be especially useful to see if the increases in

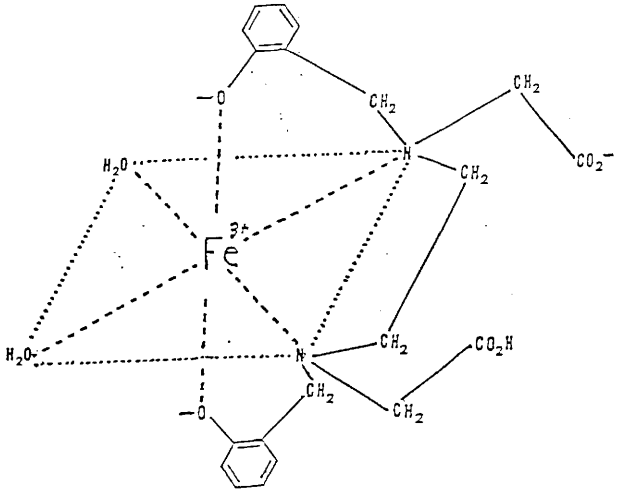


Figure 18. Proposed structure for HFeL species.

stability constants with decreases in metal ionic radii reaches a maximum at some point and then starts to decrease. This would then show the favored metal ionic radii for the cavity created by HBED when it wraps around a metal ion.

In addition to more stability constants, one important area of additional study would be proton NMR studies of complexes of HBED at various -log[D] values. These studies would indicate which coordinating groups on the HBED were in fact coordinated to the HBED and which were not, such as protonated groups in diprotonated and monoprotonated chelate complexes. The noncoordinating groups would show NMR shifts at -log[D] values near the values of the log of their protonation constants.

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